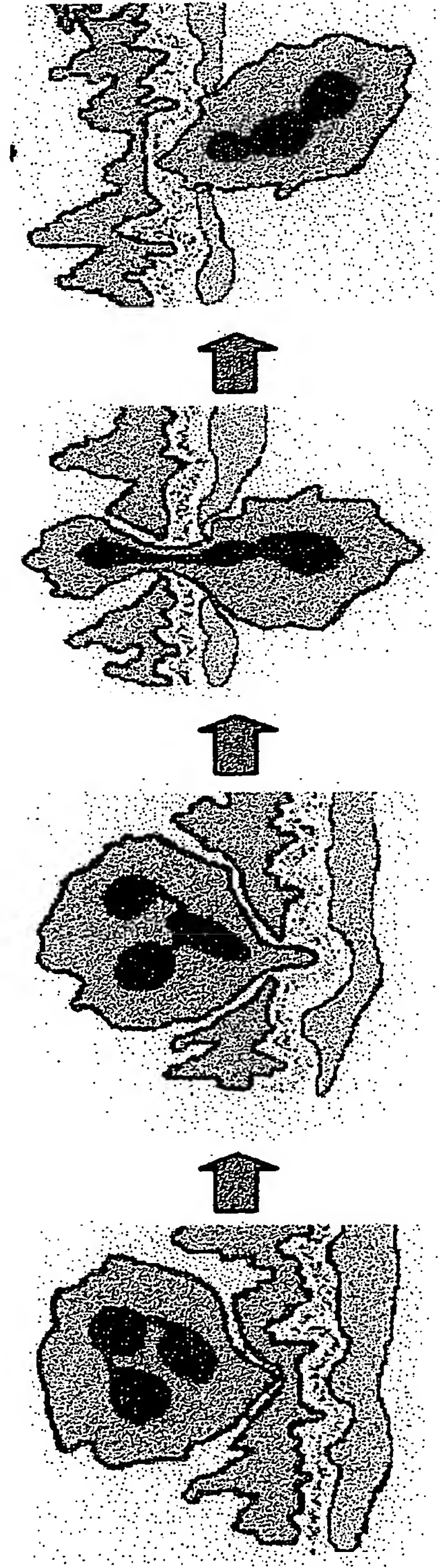
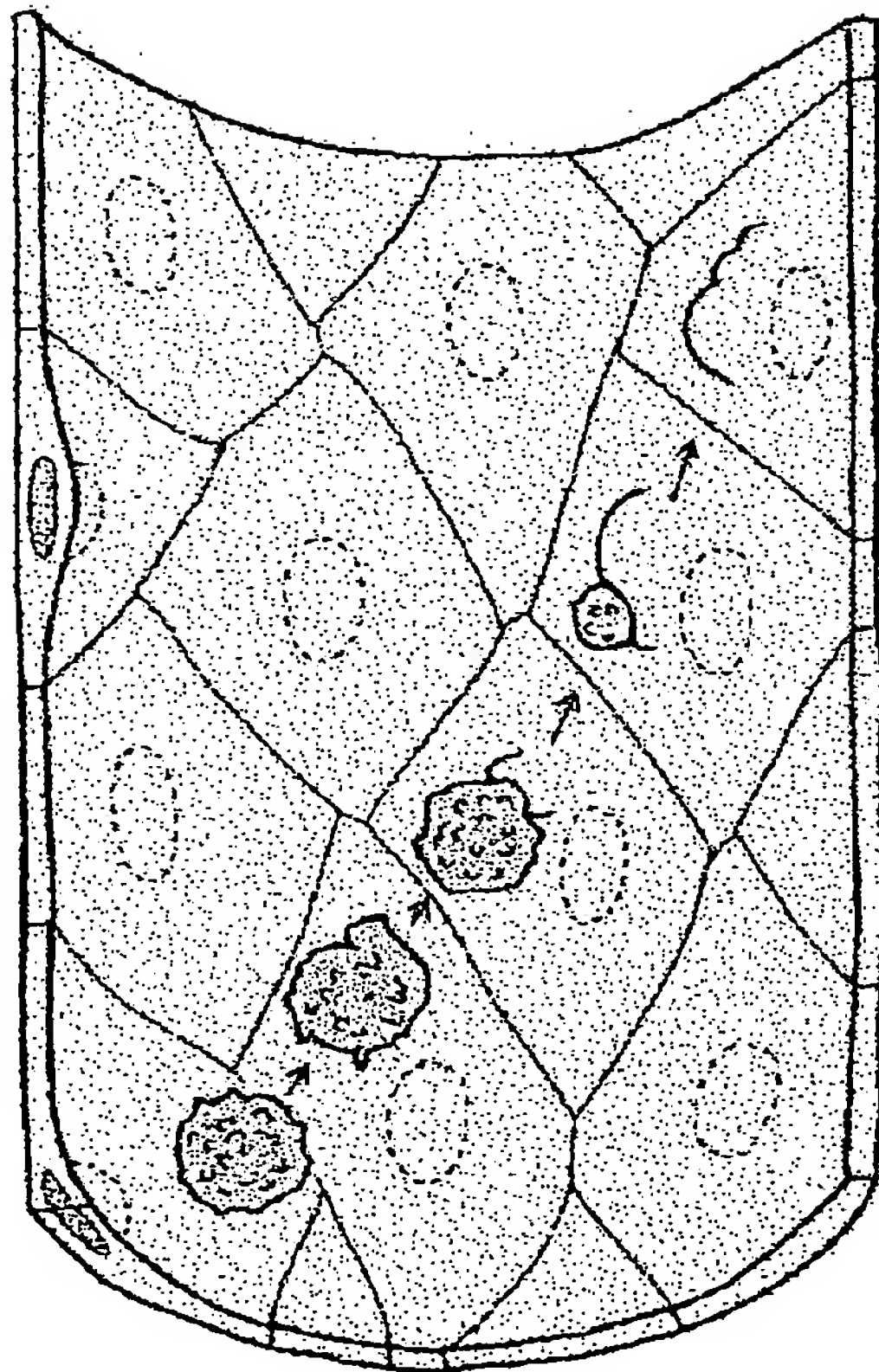


FIG. 1



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# FIG. 2

T7Select415-1b. T7Select1-1b CLONING SITE																		
CAPSID PROTEIN 10B GENE																		
aa348																		
..Met	Leu	Gly	Asp	Pro	Asn	Ser	Ser	Ser	Val	Asp	Lys	Leu	Ala	Ala	Leu	Glu		
..ATG	CTC	GGG	GAT	CCG	AAT	TCG	AGC	TCC	GTC	GAC	AAG	CTT	GCG	GCC	GCA	CTC	GAG	TAA
						BamHI	EcoRI	SacI	SalI	HindIII	NotI							

MOUSE MICROGLIA CLONE Ra2      CONFIRMATION OF INSERTED cDNA FRAGMENTS

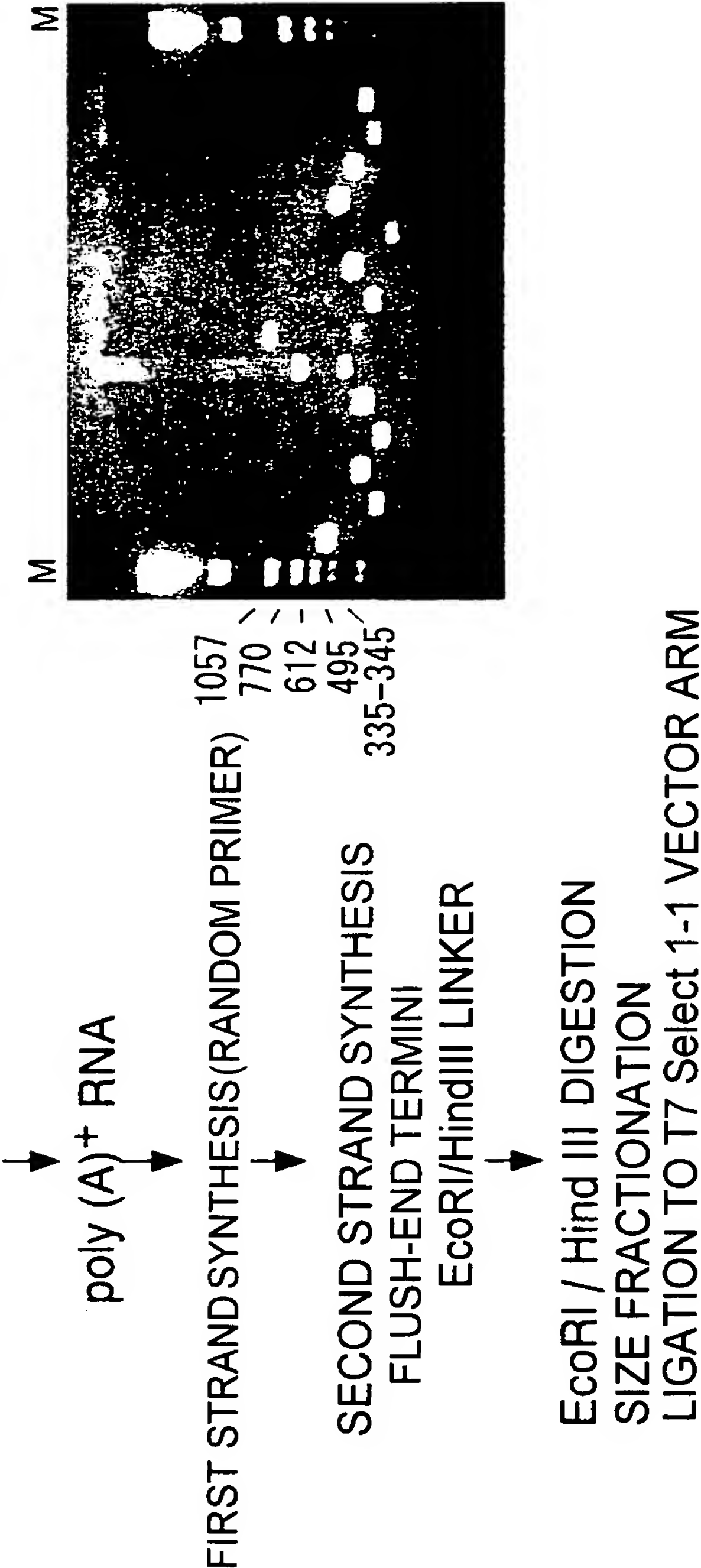


FIG. 3

T7Select415-1b, T7Select1-1b CLONING SITE

CAPSID PROTEIN 10B GENE

aa348

...Met Leu Gly Asp Pro Asn Ser Ser Val Asp Lys Leu Ala Ala Leu Glu

...ATG CTC GGG GAT CCG AAT TCG AGC TCC GTC GAC AAG CTT GCG GCC GCA CTC GAG TAA

BamHI EcoRI SacI SalI HindIII NotI

• SYNTHETIC OLIGONUCLEOTIDE -1 (84mer)

45 BASES (15 a.a.)

Asn Cys X X X X Cys Lys Leu

GCCCAGCCGGCCAAATTCNNKNNK——NNKNNKTGCAAGCTTGCGGCCGCAGGT

Mun I Hind III

• SYNTHETIC OLIGONUCLEOTIDE -2 (81mer)

45 BASES (15 a.a.)

Asn Ser X X X X Lys Leu

CAGCCGGCCTCGAATTCNNKNNK——NNKNNKAAGCTTGCGGCCGCAGGT

EcoRI Hind III

PCR AMPLIFICATION  
RESTRICTION ENZYME DIGESTION  
LIGATION TO T7Select 415-1 VECTOR ARM

↓

N: G, A, T, C MIXTURE  
K: G, T MIXTURE  
STOP CODON FREQUENCY  
1/32/CODON



# FIG. 5

## PANNING METHOD

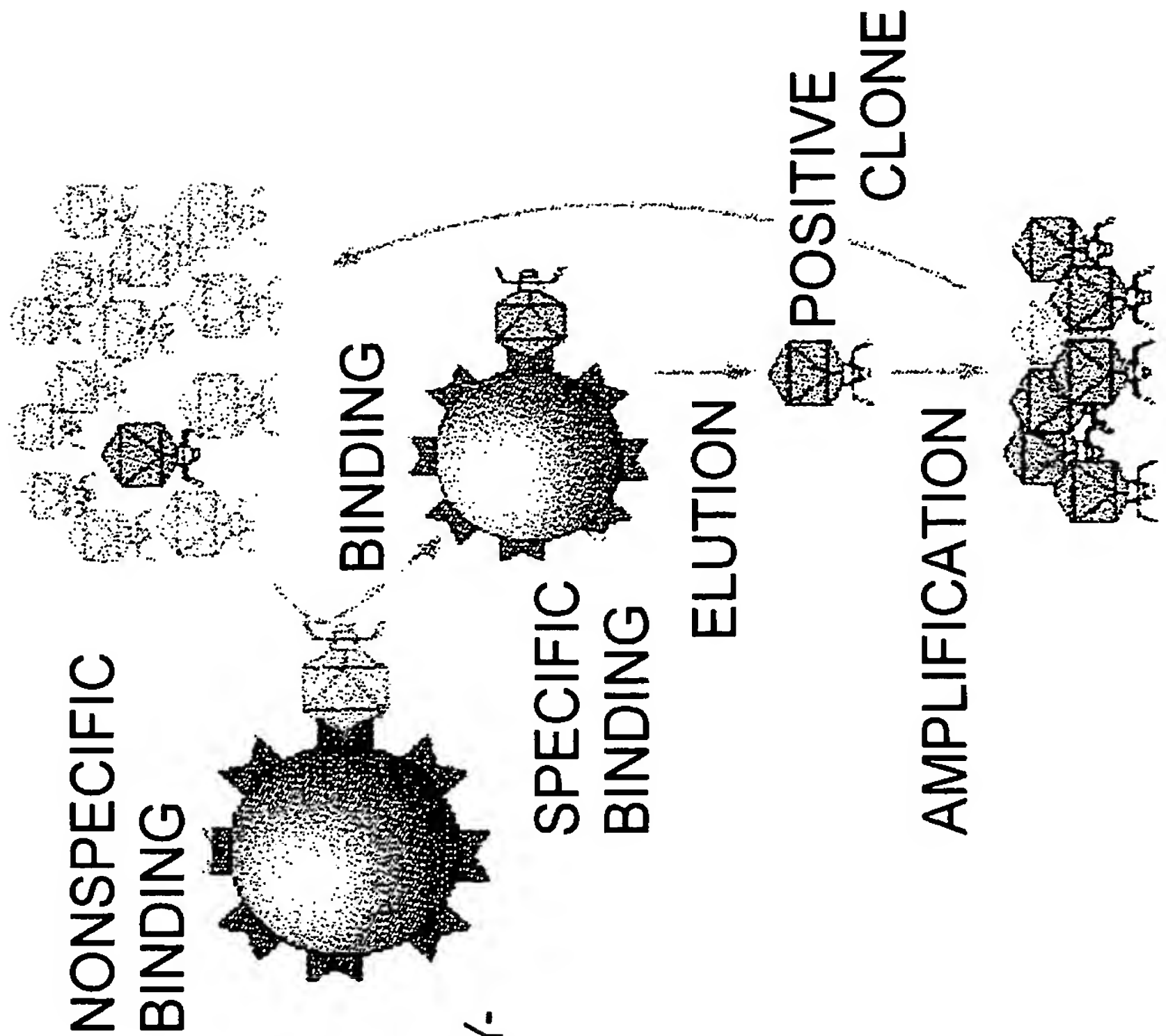
- SELECTION METHOD UTILIZING PROTEIN INTERACTION

– SELECTIVE CONCENTRATION OF CELL SURFACE-ADSORBED PHAGES

– IMPORTANT PARAMETERS

- CARRIER SELECTION
- ADSORPTION CONDITIONS
- REMOVAL OF NON-SPECIFICALLY-BOUND PHAGES
- PHAGE VS CELL
- PHAGE VS PROTEIN OR LYSATE

T7 select LIBRARY





# FIG. 6

## SCHEDULE

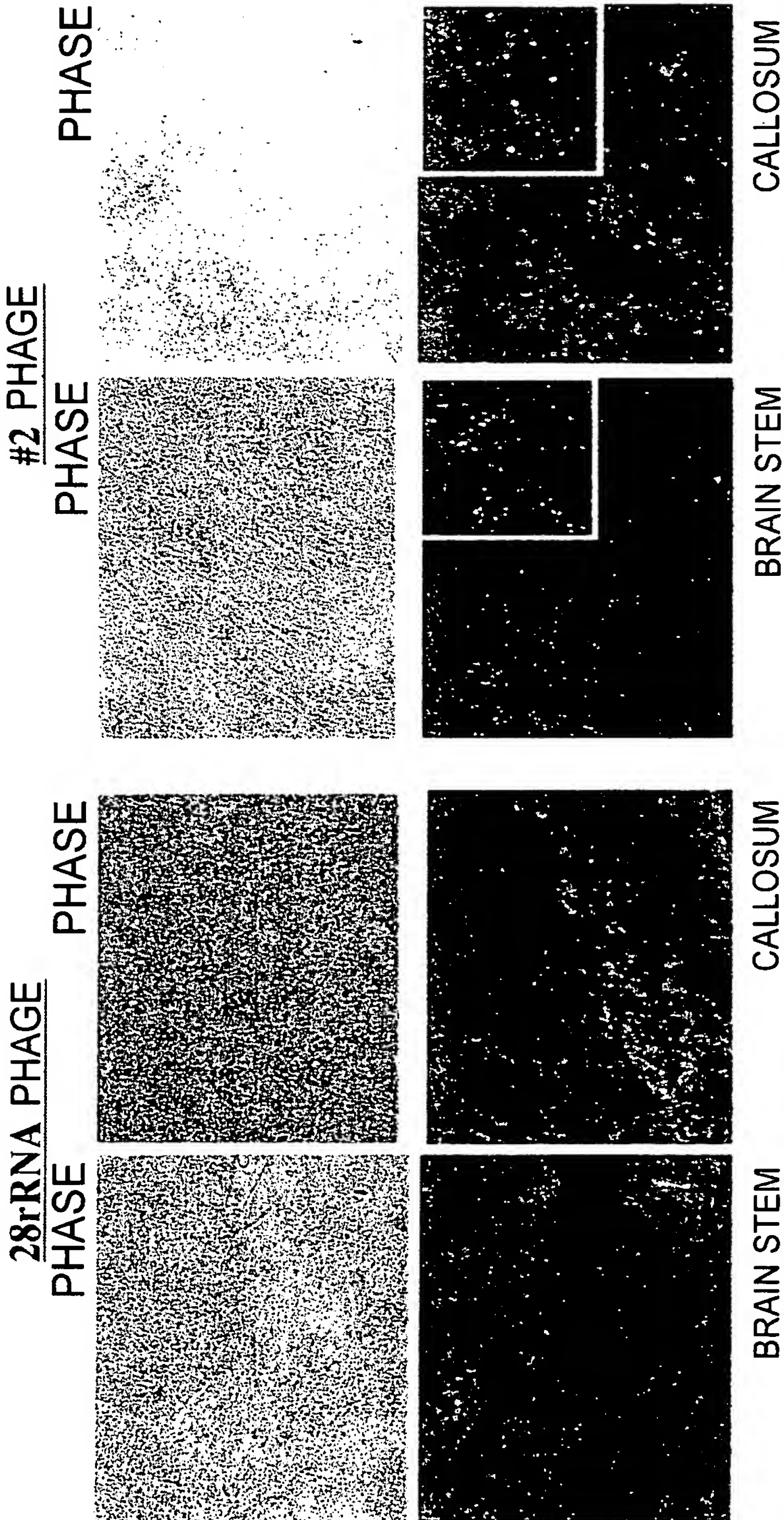
020425 : INJECTION OF CONTROL PHAGE (No.145) INTO TAIL VEIN

INJECTION OF Exp PHAGE INTO TAIL VEIN: (No.89 (SAME ARRANGEMENT AS IN #2))



5 MINUTES AFTER EUTHANASIA, 60 MINUTES AFTER EUTHANASIA

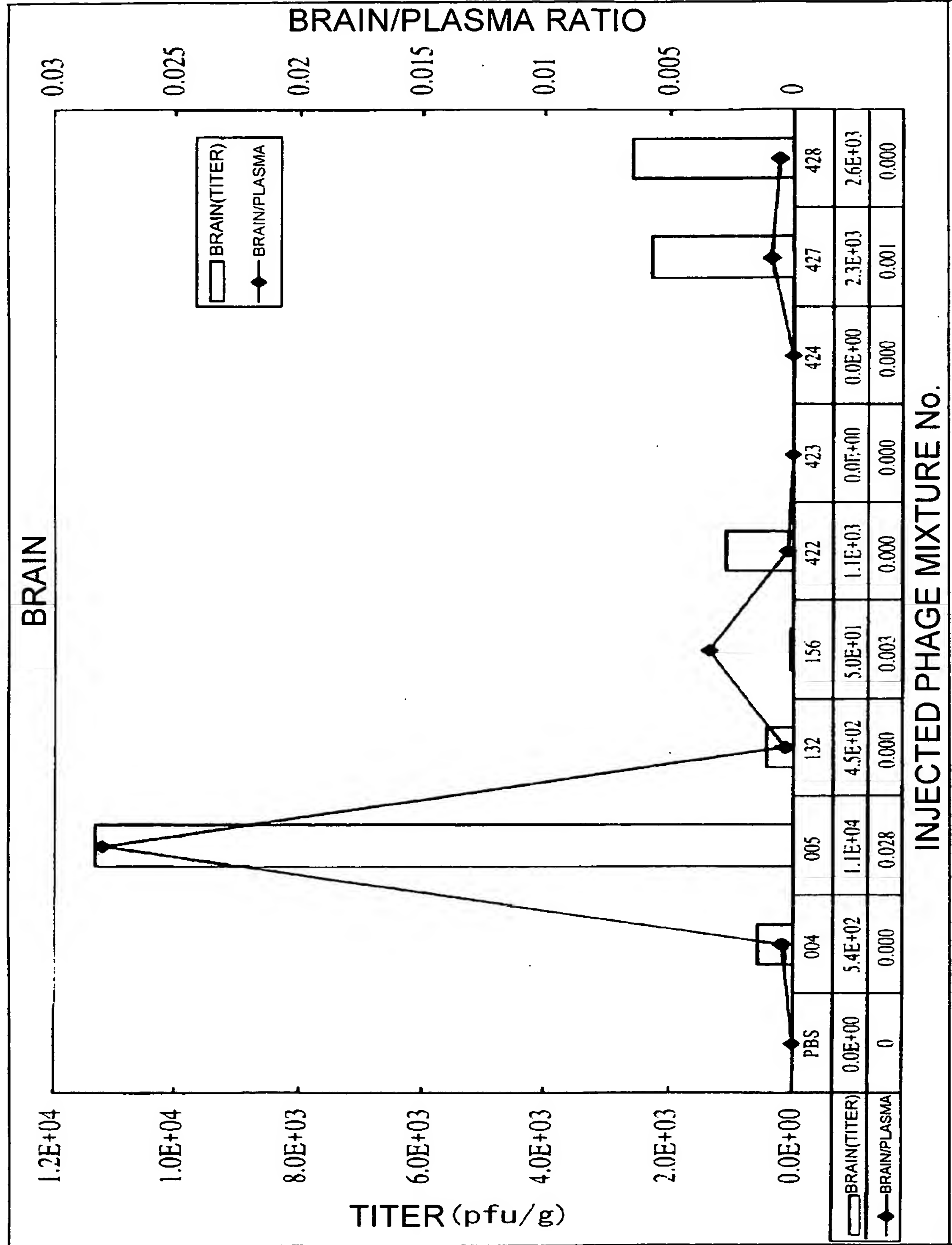
(BRAIN, LUNG, LIVER, SPLEEN, KIDNEY, TESTIS)



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Title: POLYPEPTIDES HAVING BRAIN-LOCALIZING  
ACTIVITY AND USES THEREOF (As Amended)  
Inventor(s): Makoto SAWADA  
DOCKET NO.: 084335-0201

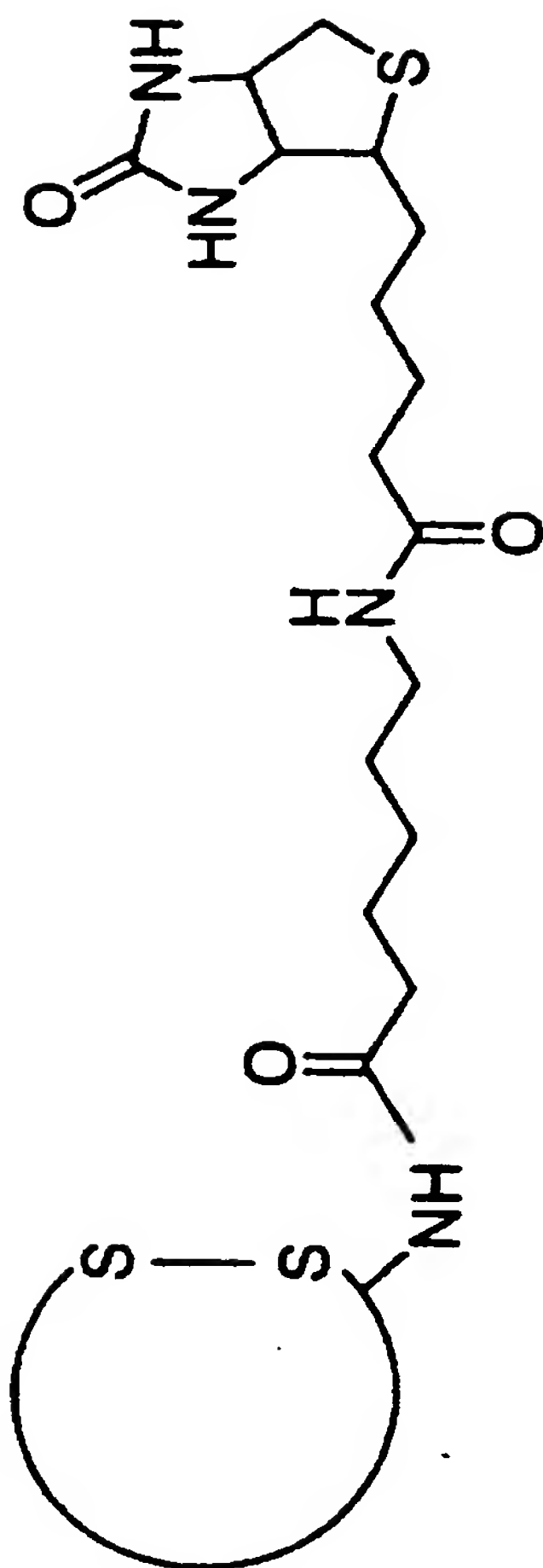
FIG. 7



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Title: POLYPEPTIDES HAVING BRAIN-LOCALIZING  
ACTIVITY AND USES THEREOF (As Amended)  
Inventor(s): Makoto SAWADA  
DOCKET NO.: 084335-0201

FIG. 8



BIOTIN



FIG. 9

CONTROL

HIPPOCAMPAL PYRAMIDAL  
CELLS (CA1-CA2 REGION)



1

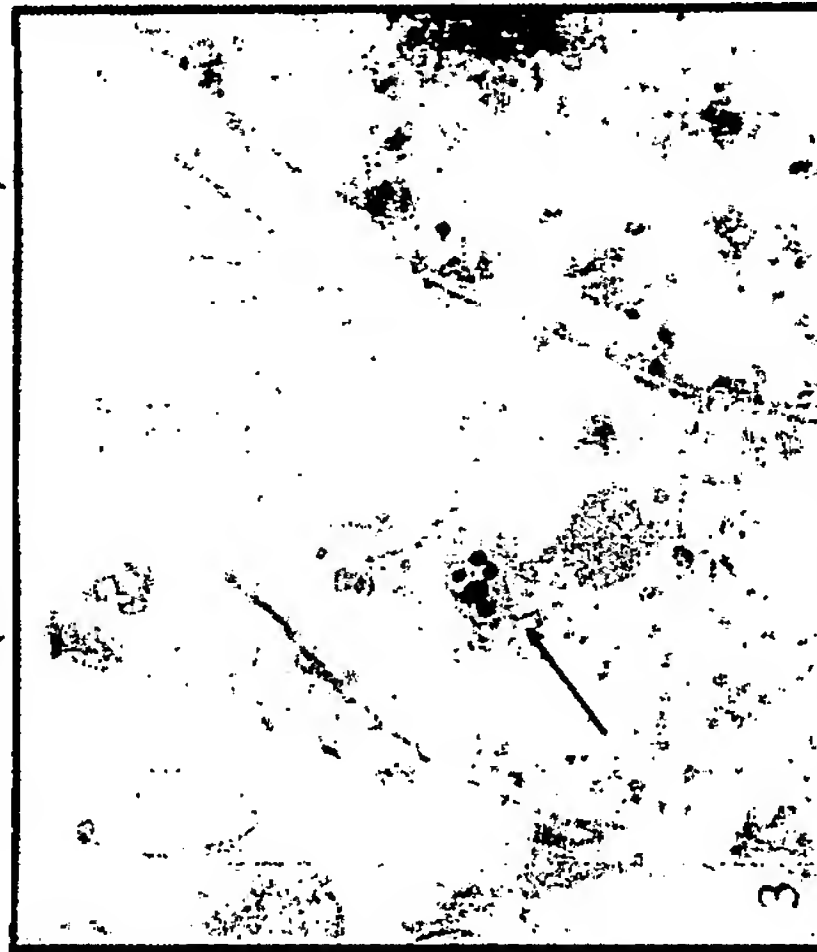
CEREBELLAR PURKINJE  
CELL REGION



2

BIOTIN -T2J002

HIPPOCAMPAL PYRAMIDAL  
CELLS (CA1-CA2 REGION)



3

HIPPOCAMPAL PYRAMIDAL  
CELLS (CA1-CA2 REGION)



5

BIOTIN -T2J004

HIPPOCAMPAL PYRAMIDAL  
CELLS (CA1-CA2 REGION)



9

HIPPOCAMPAL PYRAMIDAL  
CELLS (CA1-CA2 REGION)

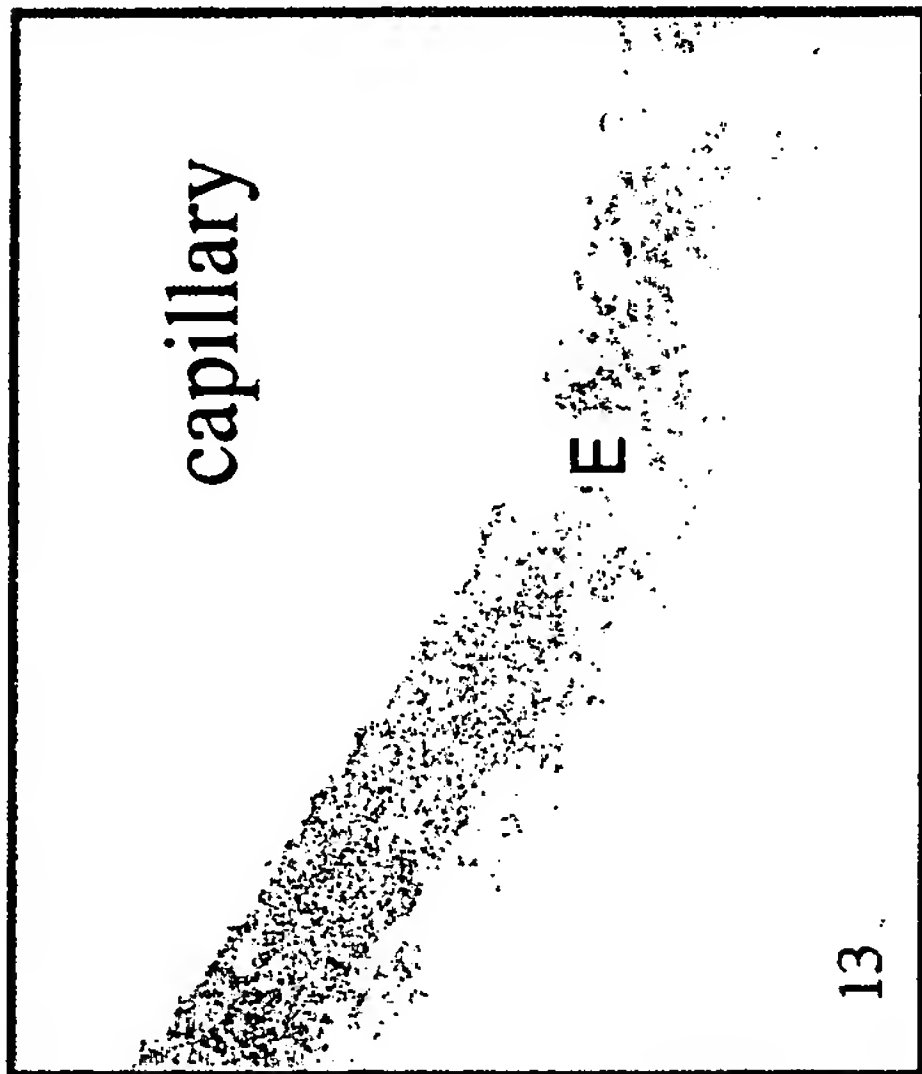


10

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FIG. 10

CONTROL  
(AVIDIN-COLLOIDAL GOLD)



13

BIOTIN -T2J002  
COLLOIDAL GOLD  
CONJUGATE



6

500nm

BIOTIN -T2J002



14

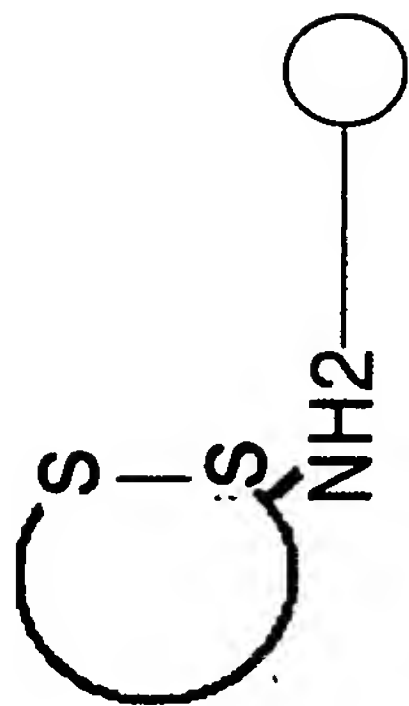
100nm

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FIG. 11

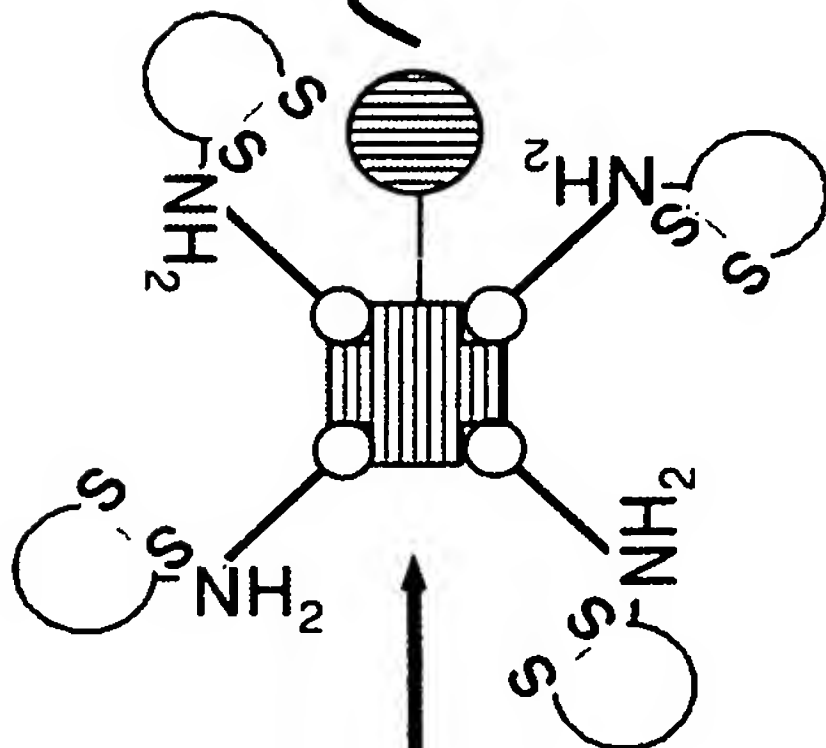
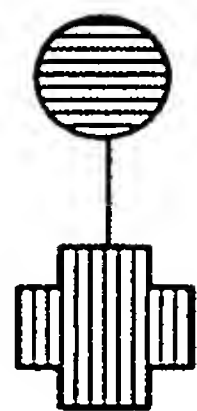
# METHOD

BIOTINYLATED CYCLIC  
PEPTIDE 004



+

AVIDIN -FITC



MBEC4

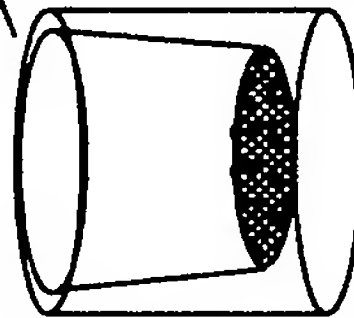
$1.26 \times 10^4$  CELLS /  $250 \mu\text{l}$

END-D

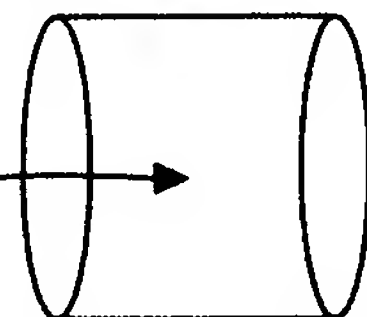
$2 \times 10^4$  CELLS /  $250 \mu\text{l}$



4 DAYS IN  
CULTURE MEDIUM



TRANSFERRED TO  
A NEW WELL



$750 \mu\text{l}$  DMEM 10% SERUM  
OR END-D MED.

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FIG. 12

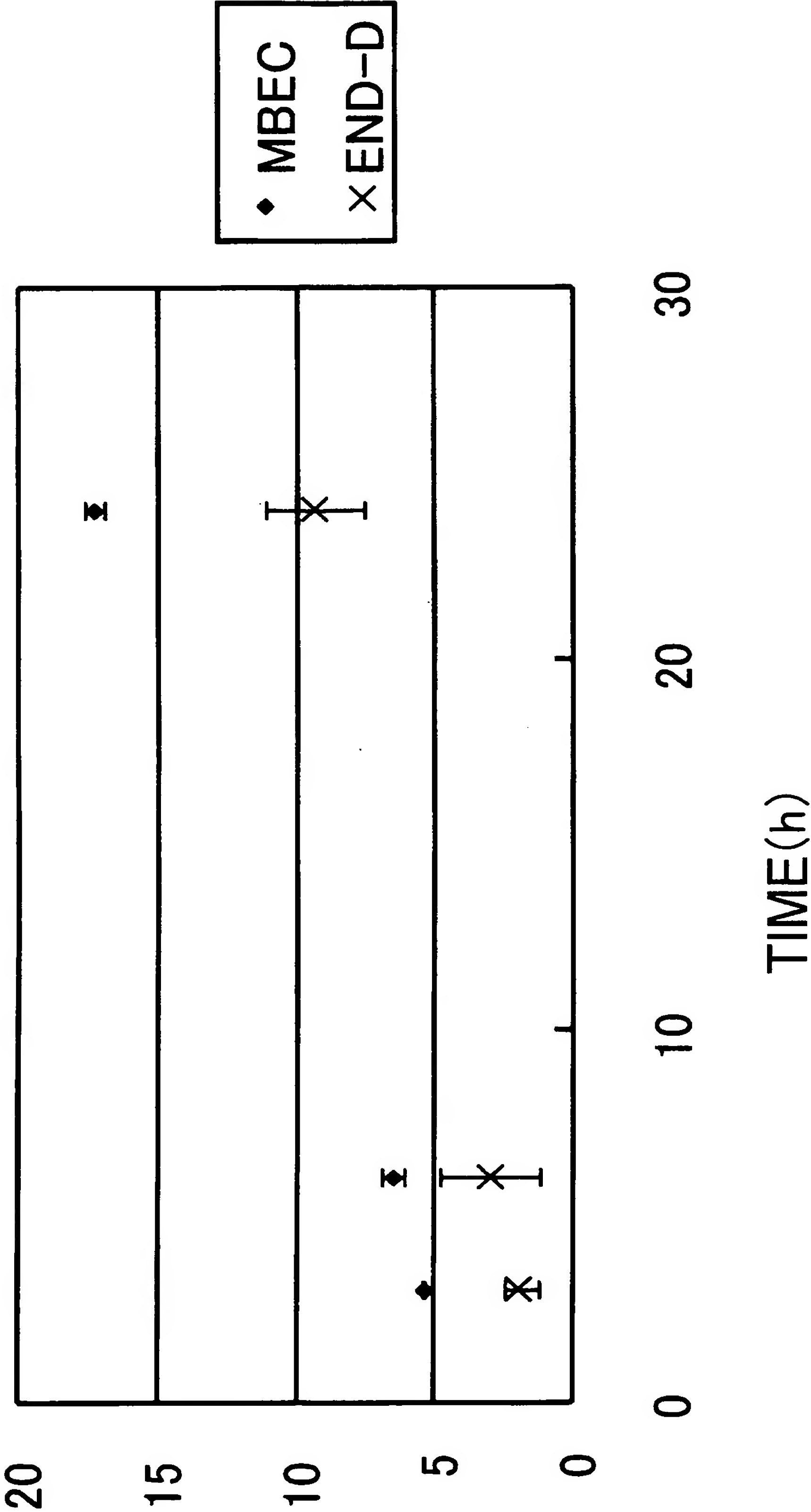


FIG. 13

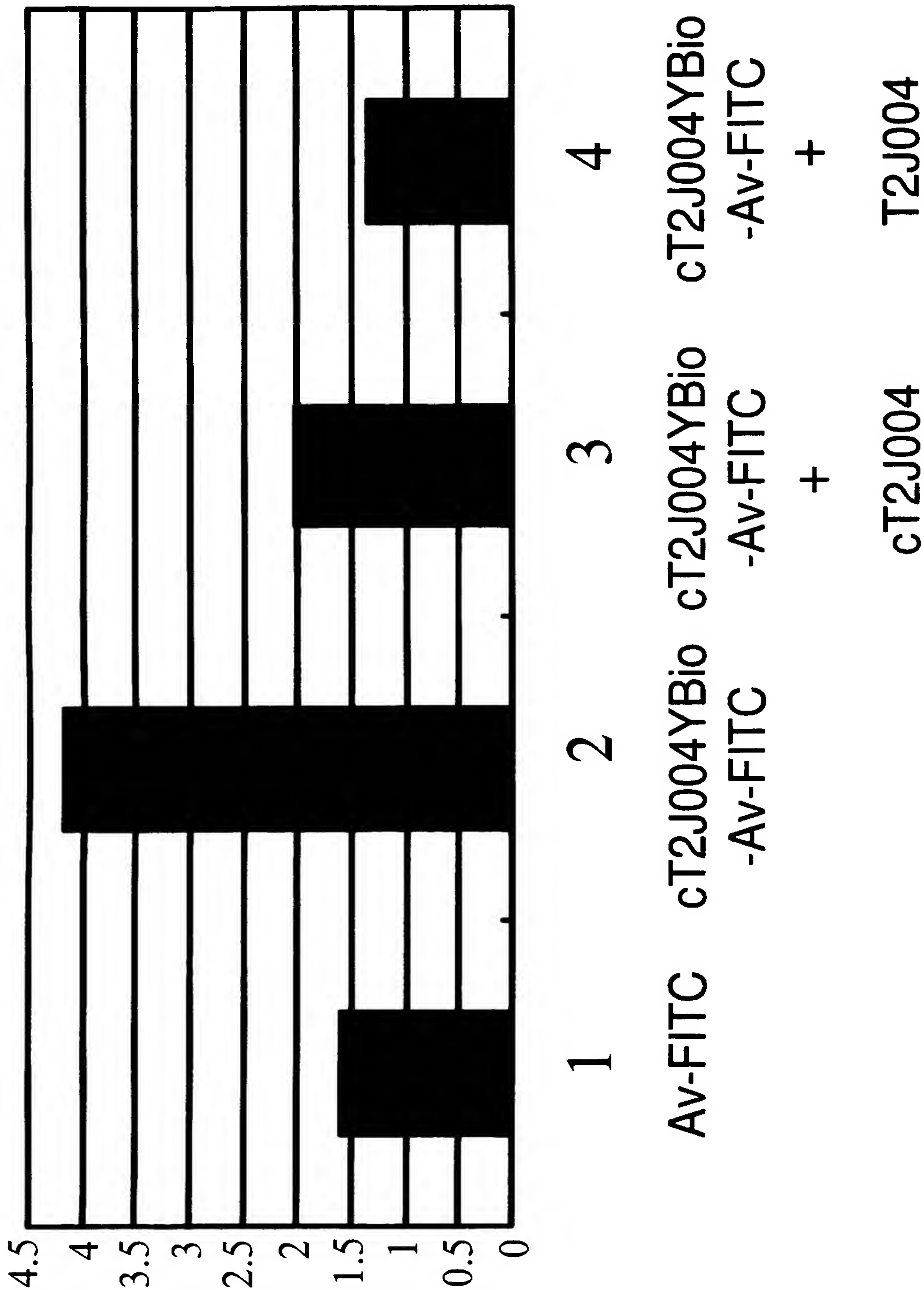


FIG. 14

METHOD

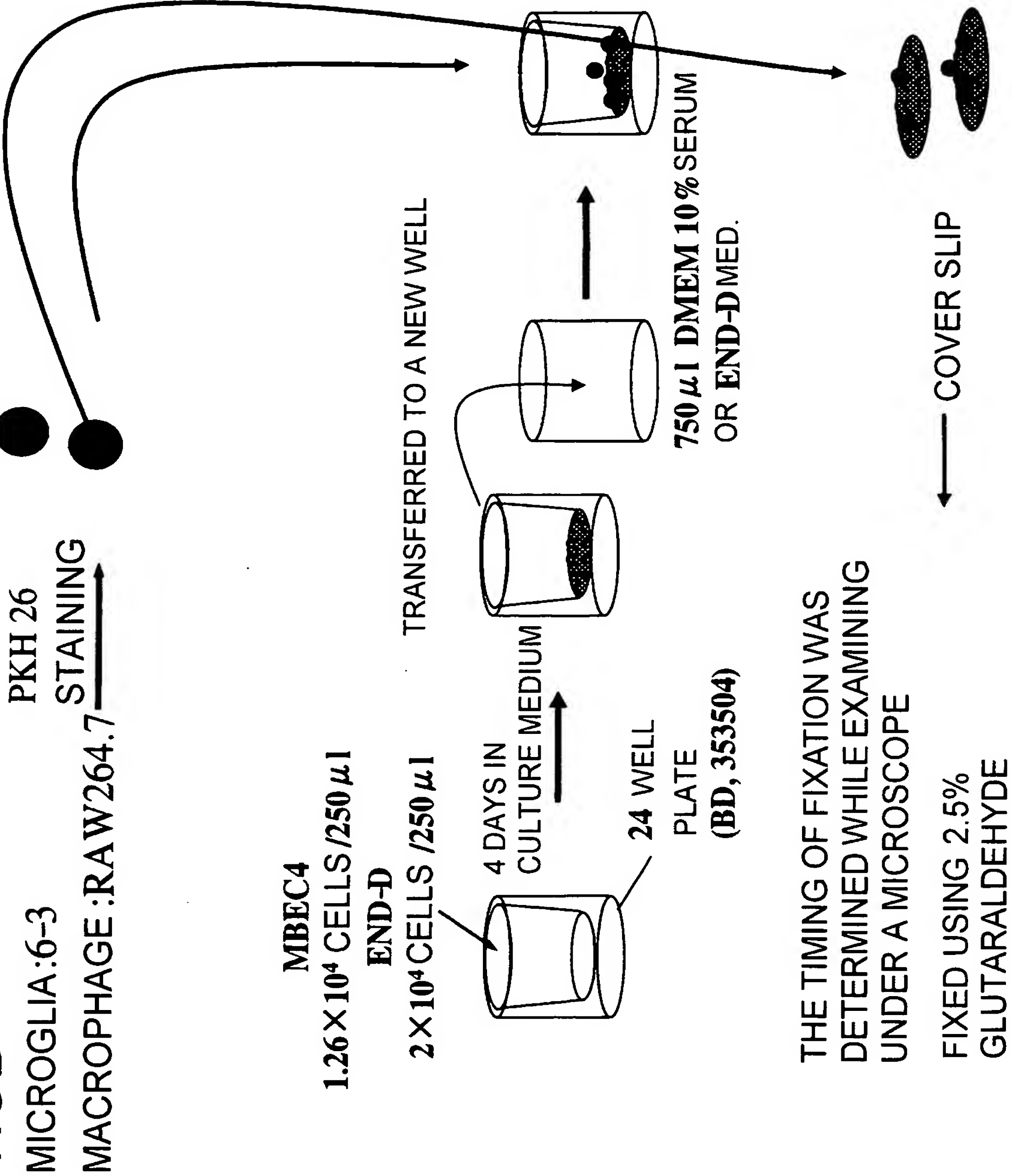




FIG. 15

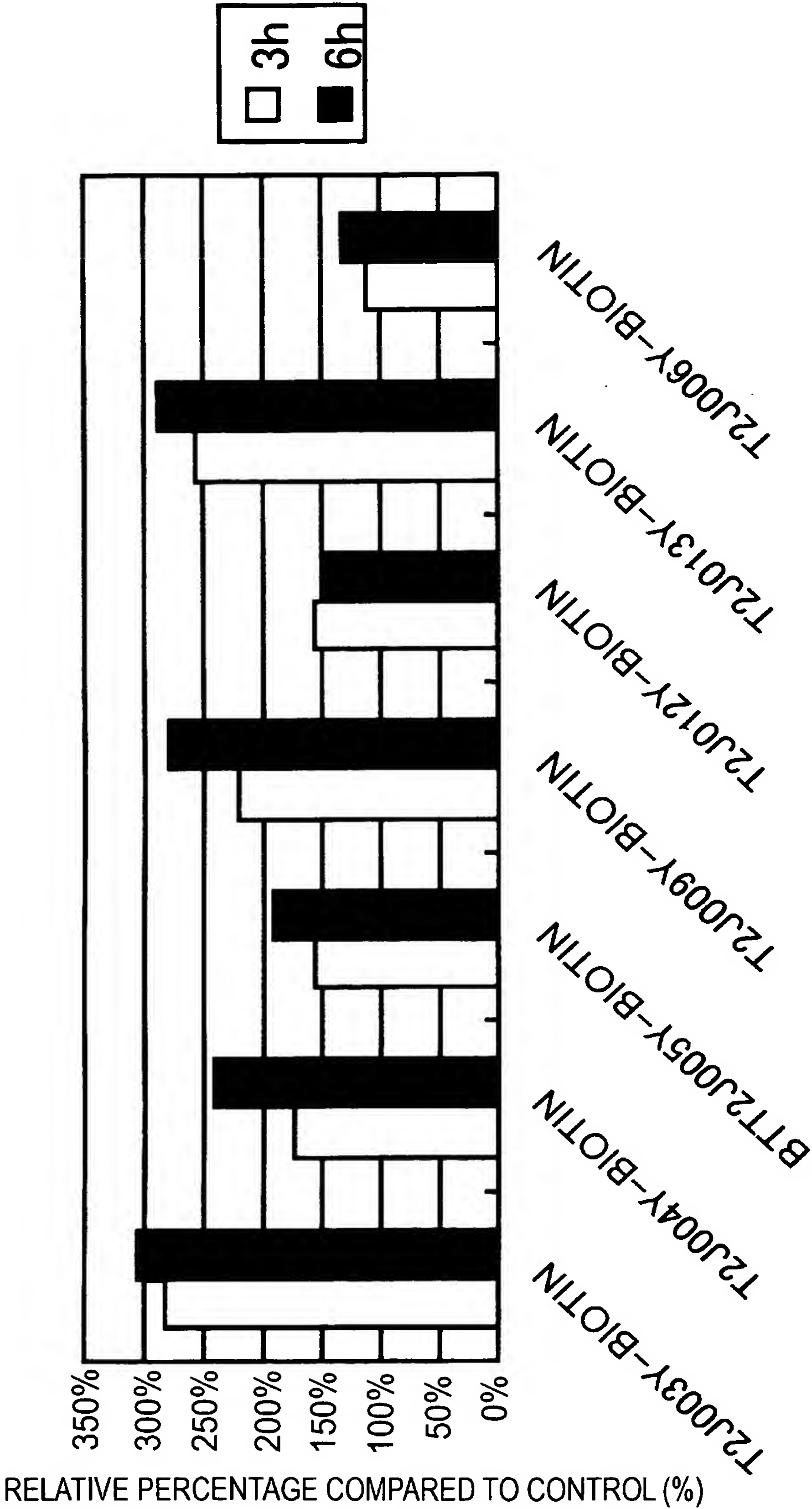


FIG. 16

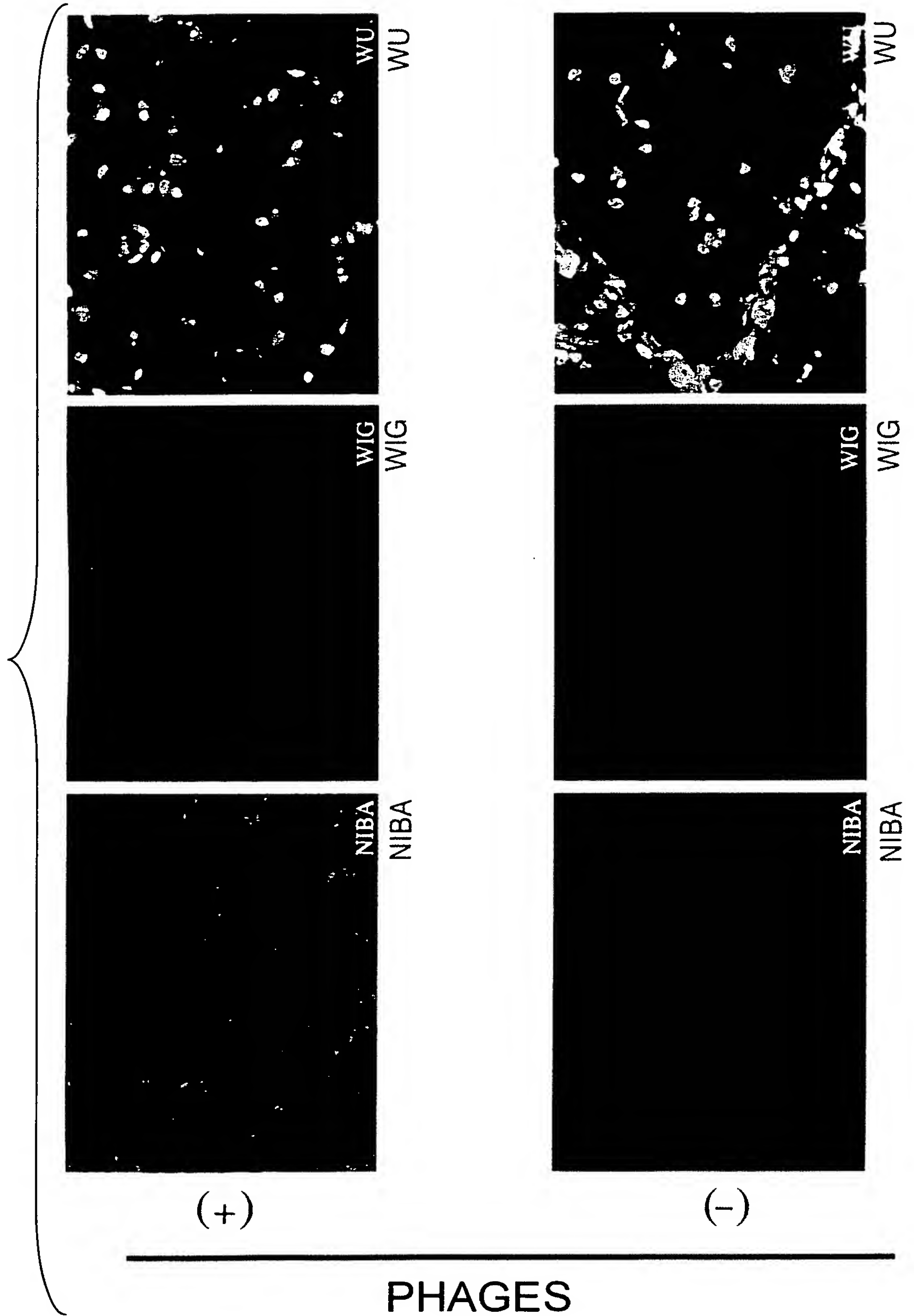
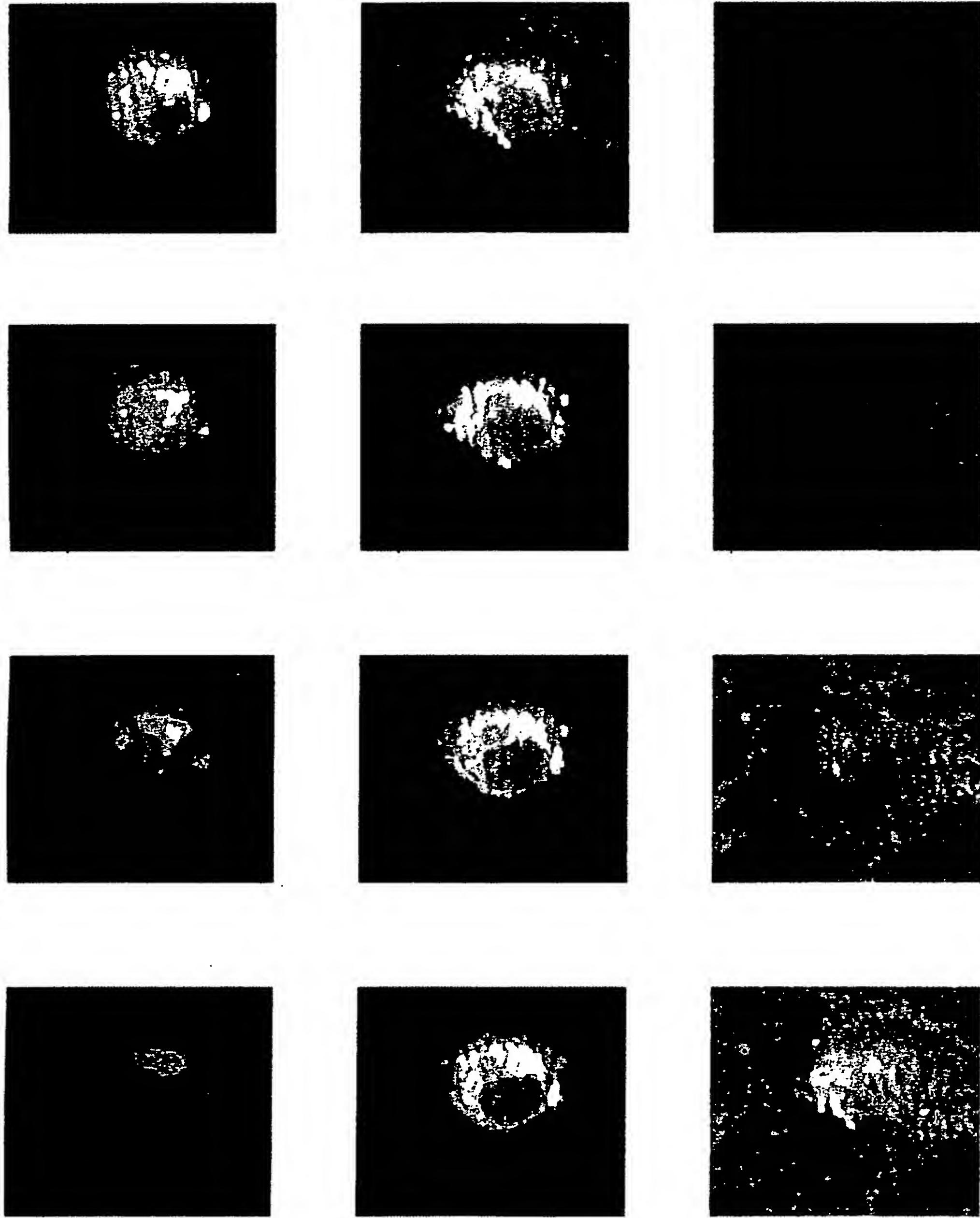


FIG. 17



GREEN:ZO-1 / RED:MICROGLIA / BLUE:NUCLEUS

FIG. 18

PHASE

PKH26-STAINED MI

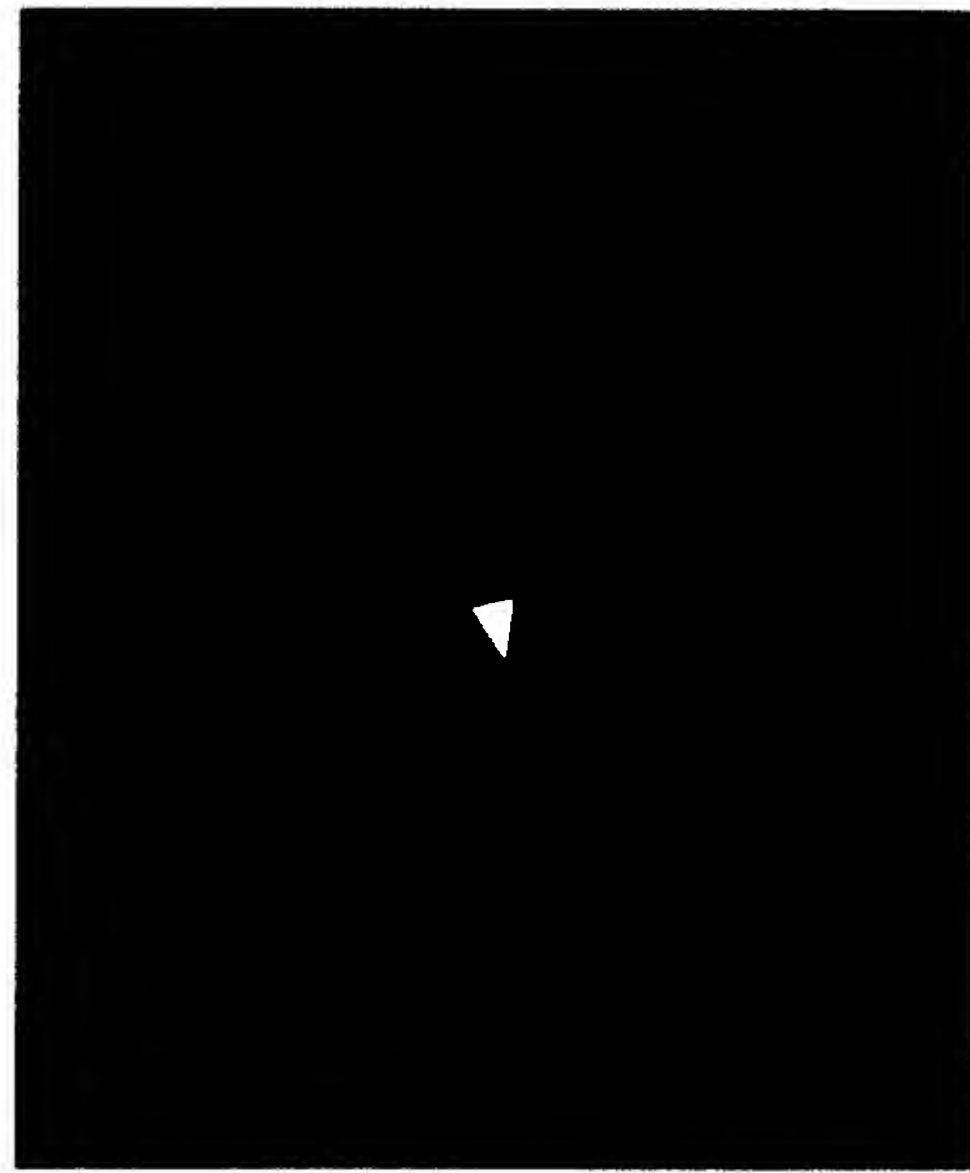
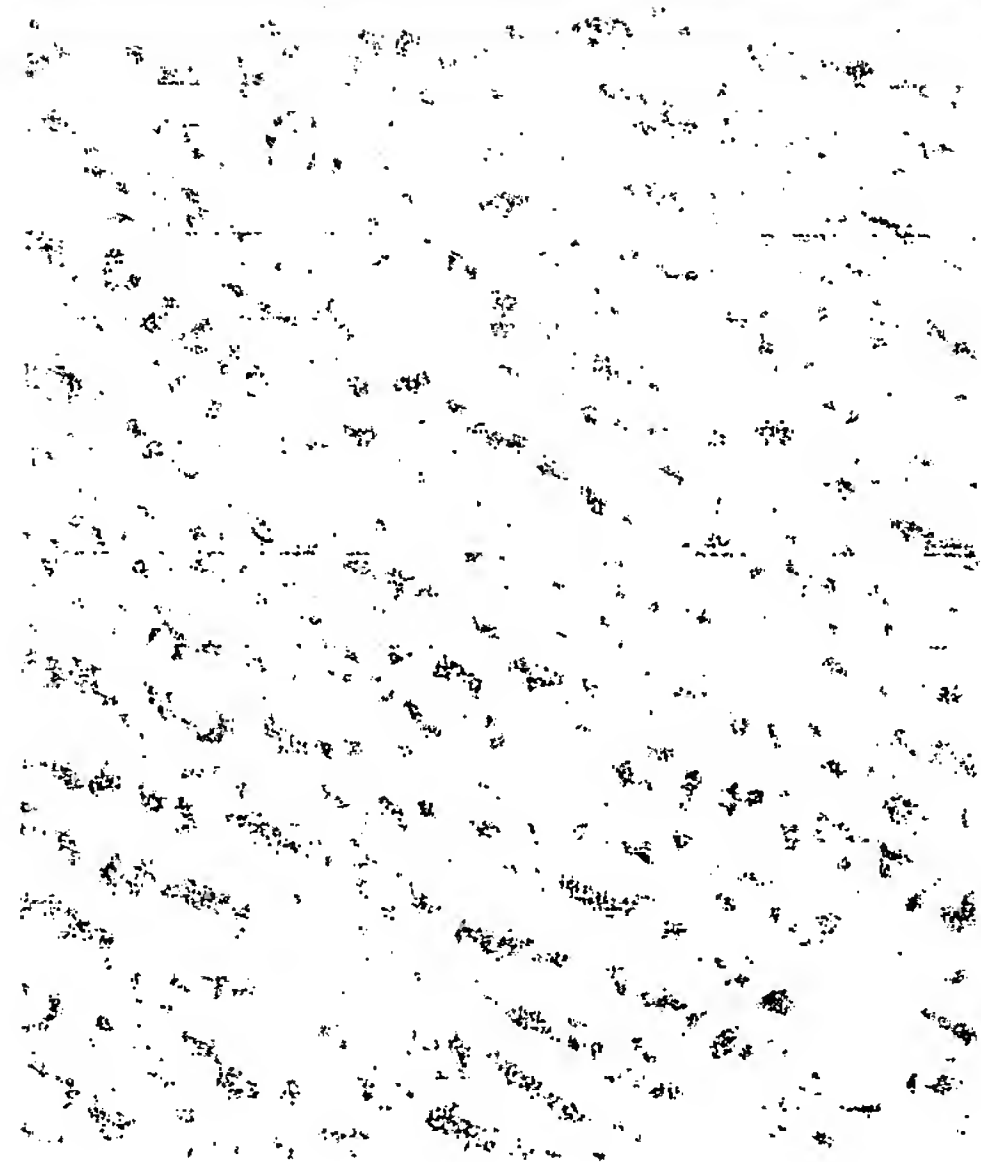
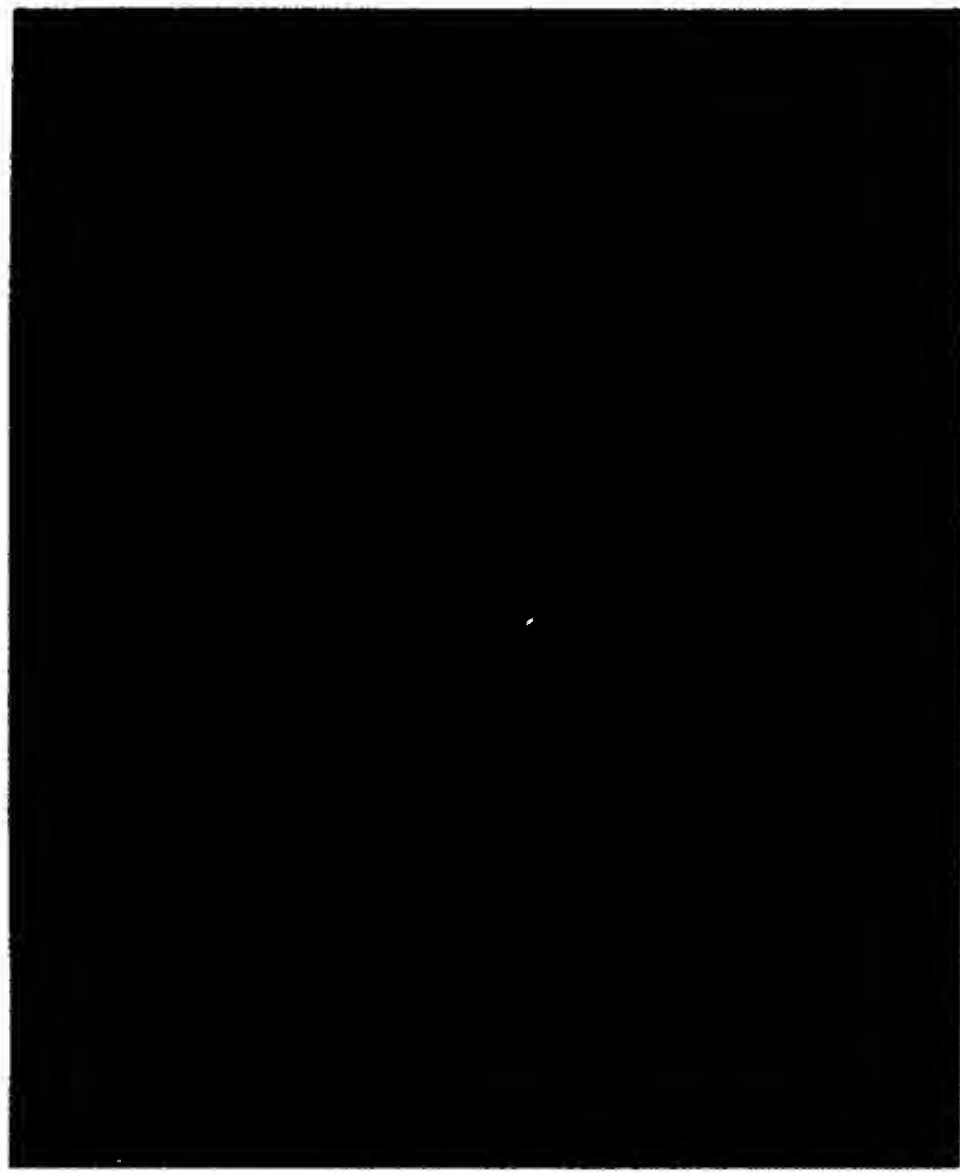
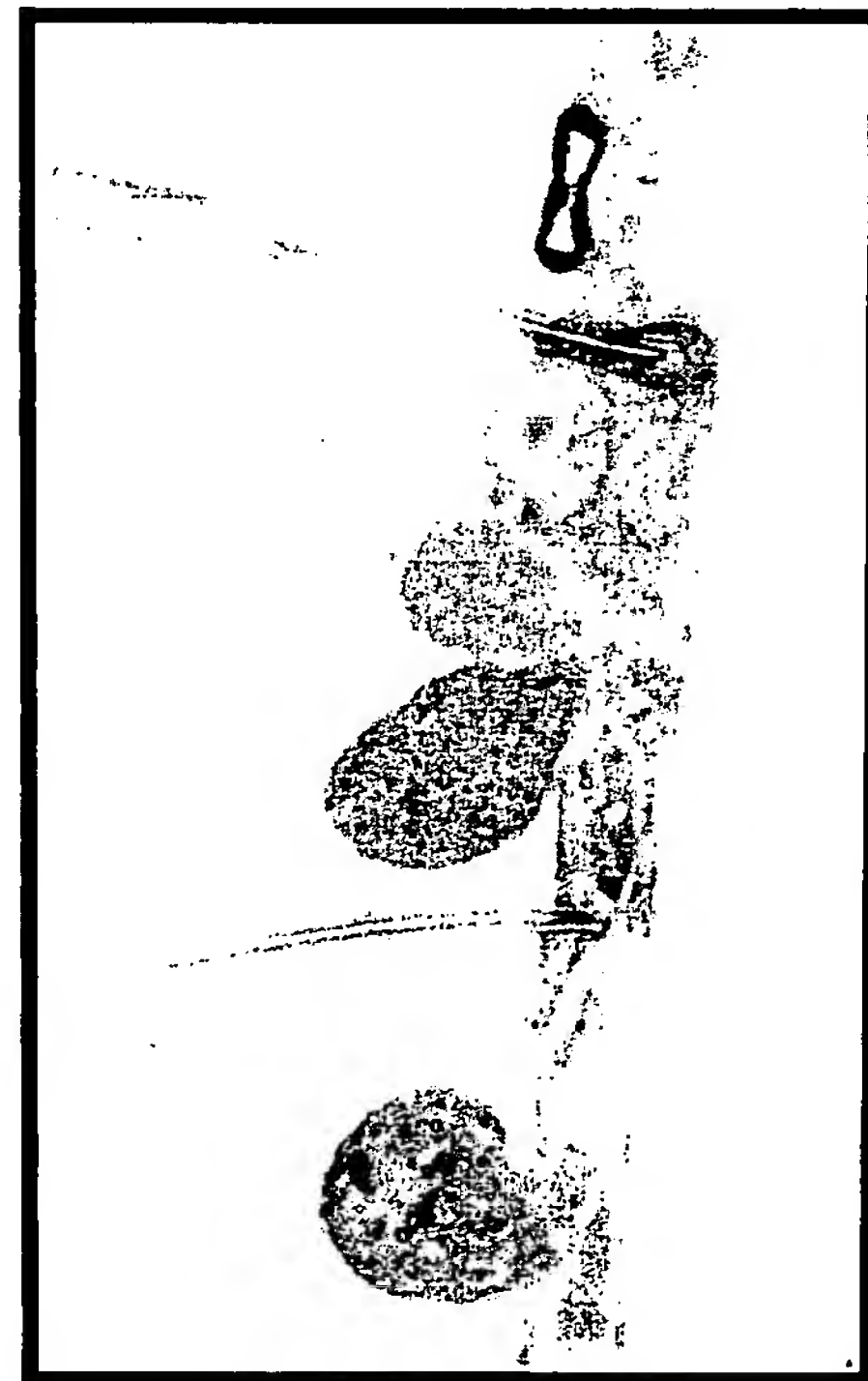
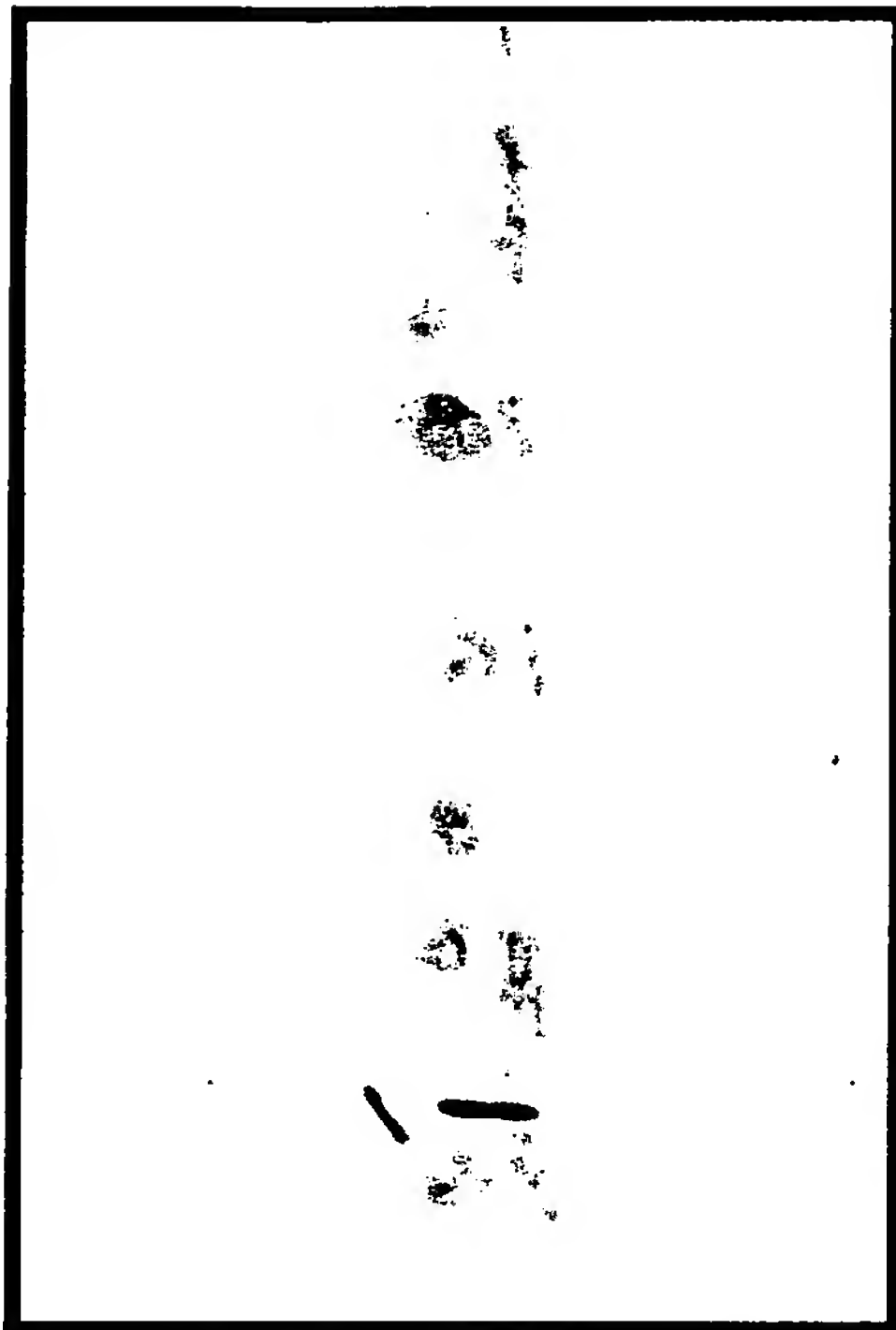


FIG. 19

MBEC4+6-3



MBEC4+Raw264.7



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Title: POLYPEPTIDES HAVING BRAIN-LOCALIZING  
ACTIVITY AND USES THEREOF (As Amended)  
Inventor(s): Makoto SAWADA  
DOCKET NO.: 084335-0201

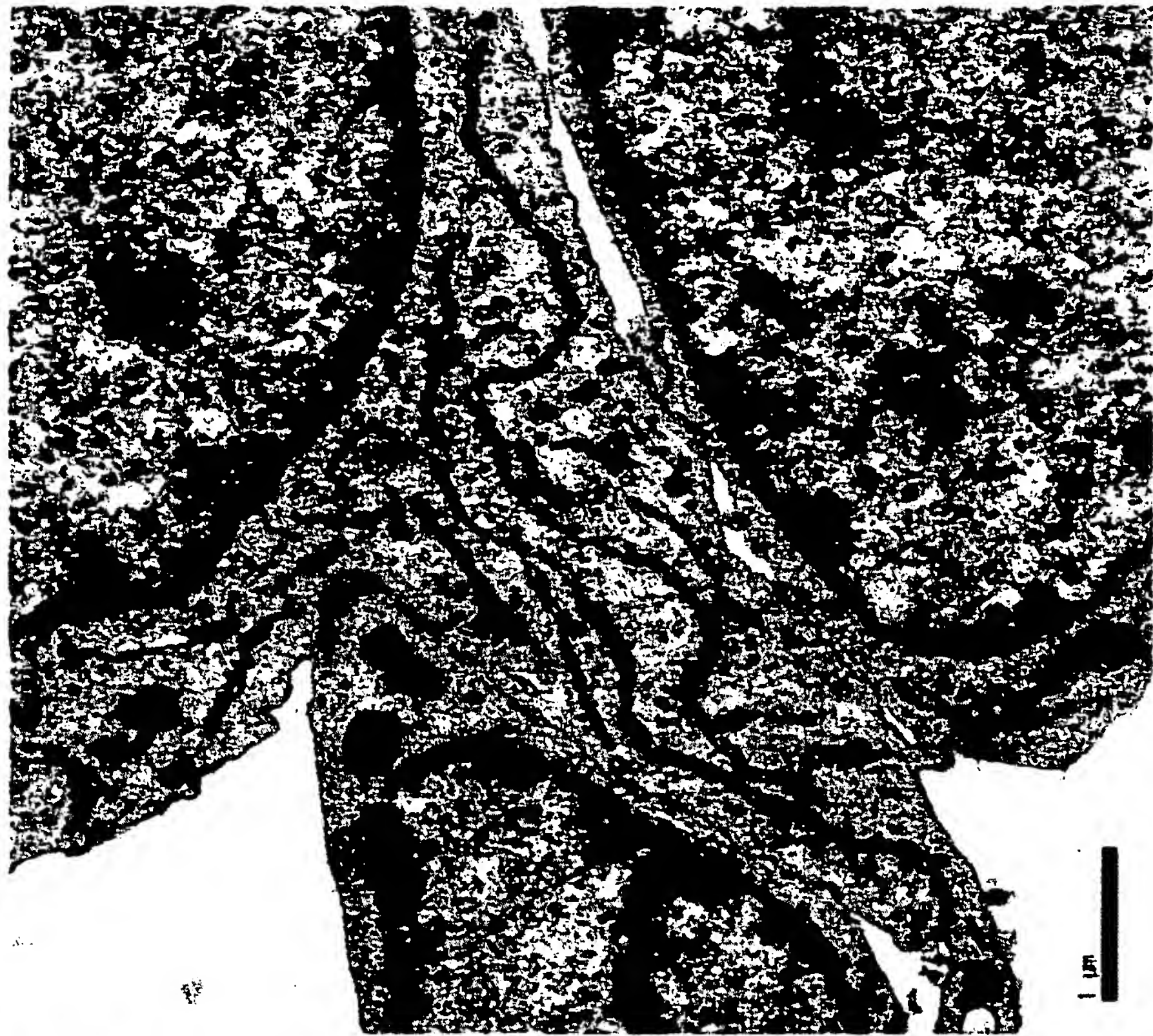
FIG. 20



0.2  $\mu$  m



FIG. 21

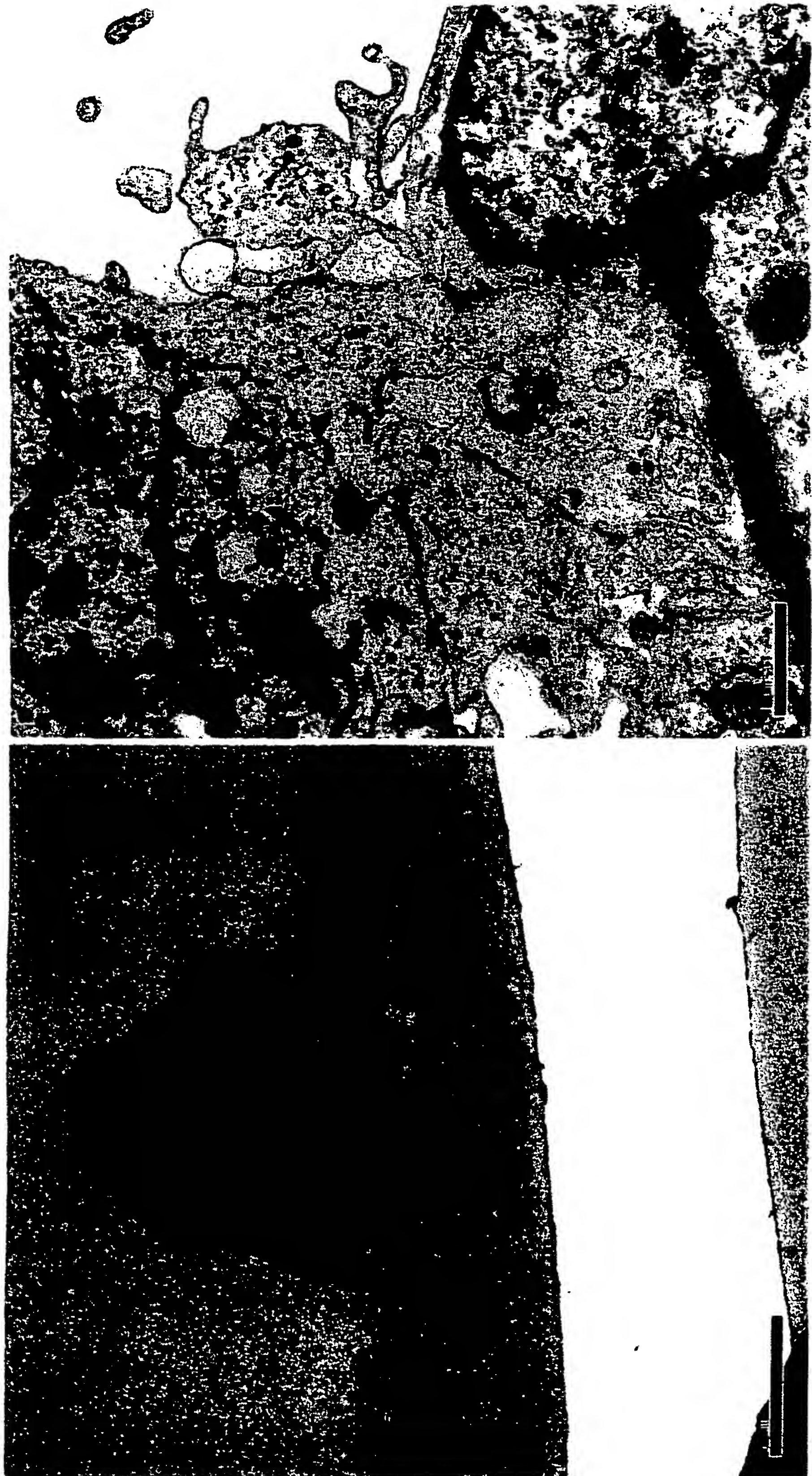


1 μm



5 μm

FIG. 22



1 μm

5 μm



FIG. 23

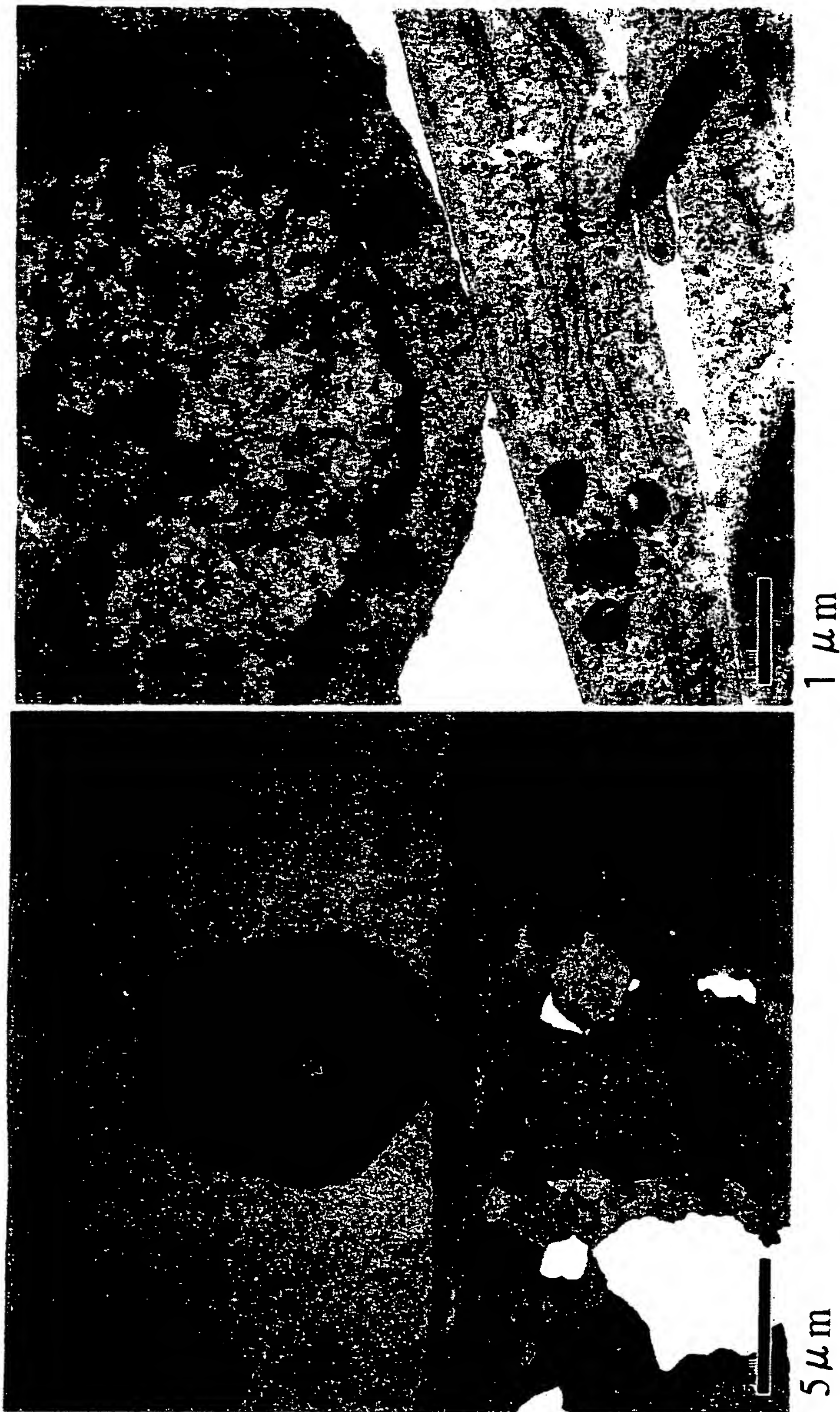
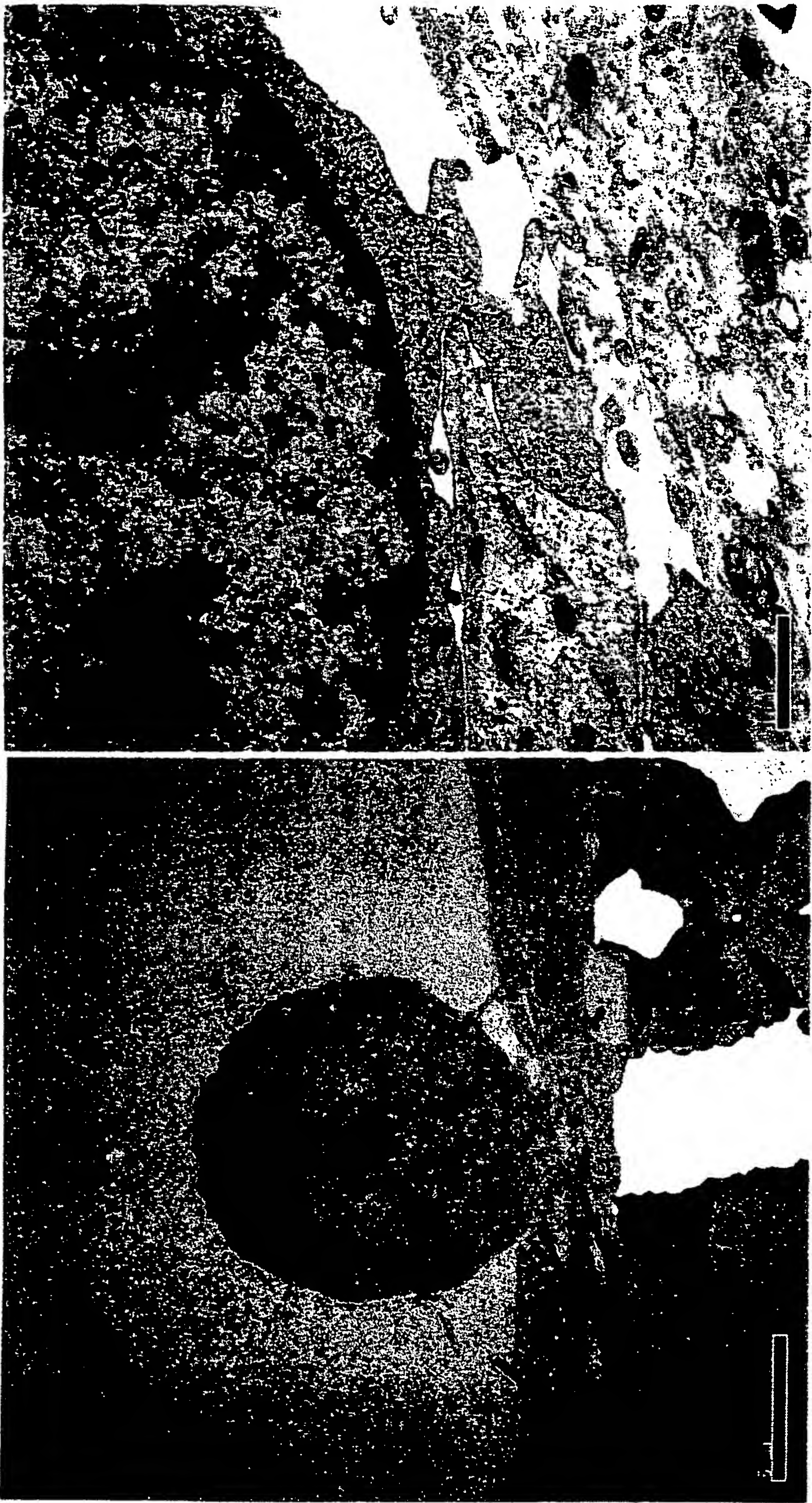


FIG. 24



1  $\mu$  m

5  $\mu$  m

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